

WARRANTY

Products are guaranteed to conform to the quality and content indicated on each vial and external labels during their shelf life. BIOTOOLS obligation and purchaser's rights under this warranty are limited to the replacement by BIOTOOLS of any product that is shown defective in fabrication, and that must be returned to BIOTOOLS, freight prepaid, or at BIOTOOLS' option, replacement of the purchasing price. Any complaint on damaged goods during transport must be directed to the handling or transport agent.

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Produced by:

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1. PRODUCT DESCRIPTION

Aliquoted ready-to-use vials or plates include BIOTOOLS DNA Polymerase, dNTPs, MgCl₂ and Reaction Buffer in gel format. This format minimises handling steps and reduces risk of contaminations; only primers and DNA template have to be added.

BIOTOOLS DNA Polymerase in gel form* represents a step forward in respect to its liquid counterpart because it is stored at 4°C, and can be shipped and handle at room temperature. Biotools offers two different formats of this product: "Ready-to-Use" Vials (96 x 0.2 ml vials) and "Ready-to-Use 96 Well Plate".

TABLE 1. Advantages of gelification technology

GEL FORM
Ready-to-use format (one tube-one reaction)
Storage at 4°C
Shipping and handling at room temperature
Saving on consumables
Open field experiments allows
Less handling steps
Experienced users are not required

BIOTOOLS DNA Polymerase is a recombinant modified form of the *Taq* DNA Polymerase expressed in *E. coli* (see **Note 1**). It is a highly thermostable DNA polymerase suitable for applications requiring a thermostable and processive enzyme capable of synthesising DNA strands at elevated temperatures in DNA amplification reactions or similar (e.g. primer extension).

*Gelification technology is covered by an international Biotools B&M Labs. patent

Product applications are:

• High throughput PCR

To perform PCR, only the addition of a template and primers is required. The BIOTOOLS DNA Polymerase gel form can be used with conventional thermal cyclers.

• Conventional End-Point PCR

2. ENZYME PROPERTIES

Enzyme concentration:	1 U/rxn
Optimal Elongation temperature	72°C
Size of PCR products:	Up to 5 Kb
PCR cloning:.....	T/A
Endonuclease activity:	No
Reverse transcriptase activity:	No
5'→3' exonuclease activity:.....	Yes
3'→5' proofreading activity:.....	No
Nicking activity.....	No

Note 1:

This enzyme is not recommended for certain experiments dealing with amplification of sequences homologous to those found in *E. Coli*.



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BIOTOOLS DNA POLYMERASE GEL FORM

REF.	FORMAT	CONTENT
10.151	12 x 8-tube strips	Biotools DNA Polymerase Gel Form -Vials
10.542	10 x 96-well plates	Biotools DNA Polymerase Gel Form -Plates
10.543	20 x 96-well plates	Biotools DNA Polymerase Gel Form -Plates

Store at 4°C

Notice to buyers/users: Some of the applications which may be performed with this product are covered by applicable patents in certain countries. The purchase of this product does not include or provide a license to perform patented applications. Users may be required to obtain a license depending on the country and/or application.

Ed.14 – July 2011

3. STORAGE CONDITIONS

Product is stable until the expiration date shown in the main product label. Store "ready-to-use" vials or plates at 4°C. Shipping as well as reaction setup can be performed at room temperature.

4. PRODUCT SPECIFICATIONS

Unit definition-One unit is defined as the amount of enzyme which incorporates 10 nanomoles of dNTPs into acid-insoluble DNA within 30 minutes at 72°C.

5. GENERAL ASPECTS OF REACTION COMPONENTS

DNA Template

The quality and quantity of the DNA template affects both the sensitivity and efficiency of the amplification. High amounts of DNA usually increase the amplification of nonspecific PCR products.

The PCR is inhibited by various compounds i.e. ionic detergents, phenol, gel loading dyes, etc. If the template contains traces of inhibitors, reduce the amount of the DNA included in the amplification reaction, or re-purify the template by ethanol precipitation and several washing steps.

Reaction Buffer and dNTPS Concentration

The buffer provided with the kit has been specially formulated to facilitate the amplification of any PCR product. It creates the appropriate stringent conditions for primer-annealing over a wide range of temperatures. Reaction buffer includes MgCl₂ to a final concentration of 2 mM.

Regarding dNTPs, the final concentration of them in reaction mix is 200 µM total (see **Note 2**).

Note 2:

The final concentration of reagents in gels is as follows: 200 µM total dNTP, 1 X Reaction Buffer, 1 U/rxn Biotools DNA Polymerase and 2 mM MgCl₂.

Primer Design

PCR primers are usually 15-30 nucleotides in length with content of 40-60% G+C residues. To avoid primer-dimer and hairpin formation the primers should not be self-complementary or complementary to any other primer present in the reaction mixture.

The annealing temperature of the primers should be similar (< 5°C variation). Length and G+C content of primers are used to predict their annealing temperature to the template DNA. The 5' end of a primer may contain mismatches between the primer and template, whereas this is not recommended at the 3' end.

For primers < 20 bases the annealing temperature is determined by the primer with the lower T_m. As starting point use an annealing temperature below 5°C of the calculated T_m. The following equation can be used to estimate the melting temperature for primers < 25 bases.

$$T_m = 2(A+T) + 4(G+C)$$

For primers with more than 25 bases, use specialised computer programs because you have to consider interaction between bases, salt concentration and other factors.

Regarding the concentration of the primers a concentration between 0.1-1 µM is recommended; use 0.2 µM as starting point of optimisation. In case of poor PCR efficiency, increase the concentration in 0.1 µM increments.

PCR Additives

In certain cases the presence of DMSO, betaine, formamide or any other PCR additives might be necessary for optimized complex PCR reactions. The provided enzyme and buffer are compatible with most PCR additives. When calculating the annealing temperature for the PCR cycling program, it is important to take into account that certain additives may decrease the melting temperature of the primers.

6. PROTOCOL OF USE

Optimal conditions must be determined for each individual experimental system.

Proceed to the Reagent Preparation Area in a laminar flow cabinet. Wear disposable gloves and use sterile and nuclease free plastic material in order to avoid contaminations and false negative results.

1. Thaw primers and DNA template on ice.
2. Calculate the number of needed reactions do not forget to include a negative control (without template DNA). **Add primers and DNA template** at the appropriate concentration to each vial or well. **Complete with nuclease free water to a final volume of 50 µl (gel volume can be discarded for volume calculations).**
3. To achieve a Hot Start effect, **not resuspend gels**. Gelified reagents will be suspended during the initial denaturation step.
4. For thermal cycler without heated lid overlay a mineral oil layer and close the vials.

Proceed to the Amplification Area

5. Program the thermal cycler according to the guide of the amplification program (see Table 2 and Section 7). Place the vials in the thermal cycler and perform the selected PCR program.

TABLE 2. Standard Amplification Program

CYCLE STEP	N° CYCLES	TEMPERATURE	TIME
Initial Denaturation	1	94°C	5-10 min
Denaturation	25-35*	94°C	5-60 sec
Annealing		T _m -5°C	30-60 sec
Extension		72°C	60 sec/1 Kb
Final Extension	1	72°C	5-15 min
Cooling	∞	4°C	∞

* Optimize the time, the temperature and the number of cycles of the PCR

7. GUIDE TO AMPLIFICATION PROGRAM

Initial Denaturation Step- Incomplete denaturation of the PCR reaction results in an inefficient first amplification cycle and low amplification yield. However, the denaturation must be kept as short as possible in order to avoid inactivation of the enzyme. For most samples 94°C for 5-10 min should be satisfactory; templates rich in G+C often require a longer step.

Denaturation Step- The PCR product synthesized in the amplification cycling is shorter than the template DNA and therefore needs a short denaturation step; 5-60 sec of denaturation at 94°C should be sufficient.

Primer Annealing Step- In general for primers < 20 bases the optimal annealing temperature is equal to the T_m of the lowest T_m primer. To find the optimal annealing temperature, you can use a temperature gradient. Start using an annealing temperature 5 °C below T_m of the primers. If primers have a high T_m a two step cycling is recommended.

Extension Step- The annealed primers must be extended at 70-75°C. The extension time depends on the size of the expected product. For Biotools DNA Polymerase we recommend 1 min for each Kb of expected product.

Number of PCR Cycles- Cycling program usually consists on 25-35 cycles. This parameter depends on the amount of starting material and the expected yield. In certain experiments, increasing the number leads to an increase in nonspecific products. You should experimentally determine the optimal number of cycles.

Final Extension Step- After the last PCR cycle the sample should be incubated at 72°C for 5-15 min. The DNA polymerase fills the protruding ends of the newly synthesized PCR products and adds extra adenine nucleotides to the 3' ends of the PCR products.

8. TROUBLESHOOTING

Problem	Cause	Recommendation
No amplification product or low yield	Missing reagent	Check the concentration and storage conditions of primers and DNA. Repeat the PCR and make sure that no pipetting errors or missing reagents occur.
	Problems with the template	Check the concentration and quality of the starting material for possible degradation. If the template is difficult e.g. rich in G+C sequences it is recommended to add additives (e.g. DMSO). Repeat the PCR with a new dilution of template, or with a new template purification.
	Problems with the primers	Revise the primers design and their storage condition. Avoid any design prone to the formation of primer dimers. Increase the concentration of primers in 0.1 µM increments. Check primer degradation on a denaturing polyacrylamide gel.
	Incorrect PCR cycling conditions	Check the following parameters of the amplification program: Denaturation: increase the temperature and length of initial denaturation. Annealing: optimise the annealing temperature and time (see section 7). To increase the specificity you can use a touchdown or a stepdown program. Extension time- Increase the extension time by increments of 30 sec. Cycle number- Perform additional cycles by increments of 5 cycles. Verify the final elongation step.
	Mg ²⁺ concentration too low	Increase the concentration of Mg ²⁺ by adding MgCl ₂ solution in 0.25 mM increments.
Multiple non specific amplification products or background smear	Annealing temperature too low	Increase the annealing temperature by increments of 1°C.
	Problems with primers	Revise the primer design and the primer condition. Decrease primer concentration by increments of 0.1 µM. Check primer degradation on a denaturing polyacrylamide gel.
	Too much DNA template	Use dilutions of your template.
	Carry-over contamination	Always prepare a negative control PCR (no template). If the NC shows a PCR product or a smear exchange all the reagents.
	Low specificity	To increase the specificity you can perform a touchdown or step-down PCR. Reduce the number of cycles.
PCR products in negative control	Carryover contamination	Exchange all reagents.

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